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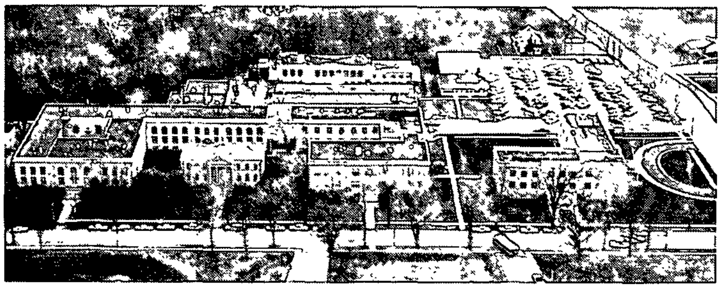

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# PLANT GROWTH: THE ROLE OF POLYAMINES

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# Plant Growth: The Role of Polyamines

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Plant Growth: The role of polyamines

Polyamines are a group of chemical compounds that are ubiquitous in nature, being found in bacteria, plants and animals. It has been reported that Leeuwenhoek observed microscopic crystals of polyamines in biological preparations during the 17th century, but only recently have their occurrence, role and importance been appreciated. Early studies correlated high intracellular levels of polyamines with periods of increased growth in cultures of bacteria, and these findings were soon extended to plant and animal species. High levels of polyamine biosynthesis are observed in rapidly proliferating cancer tissues, for example. Perhaps more exciting, however, is the relationship between polyamines and development or differentiation. Evidence is accumulating which suggests that polyamines are involved in the organized growth of tissues, organs and embryos. Inhibition of polyamine biosynthesis leads to the failure of embryo development in plants as well as animals, suggesting that they play an important role in developmental processes. Polyamines may be among the factors which control or modulate the development of a complex multicellular organism from an egg or single cell.

The polyamines (putrescine, spermidine and spermine) are derived from several amino acid precursors (figure 1). Putrescine is enzymatically formed from the amino acids ornithine and/or arginine in plants and bacteria, while only ornithine serves as the precursor in animal tissues. Spermidine and spermine are subsequently formed from putrescine by the stepwise addition of aminopropyl groups arising from s-adenosyl methionine (SAM), which is found in all cells. In certain plant tissues polyamines are found not only as free molecules, but also conjugated to phenolic acids such as cinnamic acid and ferulic acid. Nearly 90 percent of the polyamines found in tobacco flowers, for example, are present as conjugates. The significance and function of these polyamine-phenolic conjugates is not fully understood at present. Until relatively recently, only correlative types of experiments could be carried out, in which relative polyamine levels were related to growth rate or stage of development. A number of enzyme inhibitors have been developed by the Merrell Research Institute which block the formation of polyamines. Difluoromethylarginine (DFMA) and difluoromethylornithine (DFMO), amino acid analogs, specifically and irreversibly inhibit the enzymes arginine decarboxylase (ADC) and ornithine decarboxylase (ODC), respectively (figure 1). Using these compounds, polyamine levels have been experimentally manipulated to show their role in the metabolism of the tissue. Studies of this type are the most exciting and suggest a major if not essential role of polyamines in plant growth and development.

High intracellular levels of polyamines have been related to the growth of bacterial, plant and animal cultures. Mutant _E. coli_ bacteria which have a reduced capacity to synthesize polyamines exhibit slower growth than wild type cells. In selected animal cell cultures, near linear correlations have been established between growth rate and spermidine levels in the cells. High levels of polyamines have also been noted in regenerating mammalian liver. Depletion of cellular polyamines in animal tissues using inhibitors of their synthesis lead to reduced levels of DNA synthesis and cell proliferation. DFMO, the inhibitor of ODC which is principally responsible for putrescine biosynthesis in animal cells, may in fact be useful in the treatment of various types of cancerous tumors which exhibit elevated levels of polyamines.

Polyamines affect a number of aspects of cell metabolism, many of which could lead to increased growth rates. Protein synthesis is increased by polyamines, and polyamines are a standard addition to _in vitro_ protein synthesis systems. Polyamines may make initiation and elongation of the growing polypeptide chain more efficient. In cultured Helianthus tissues, auxins added to the culture medium induce protein synthesis. This elevation in protein synthesis is highly correlated to cellular polyamine levels, which rise due to auxin stimulation. Polyamines have also been shown to enhance synthesis of nucleic acids, primarily through effects on DNA and RNA polymerases. Most of the studies involving effects on nucleic acid biosynthesis have been done using animal tissue, however, and these findings need to be confirmed in plant systems. On a molecular level, it has been shown that polyamines are capable of binding across the major and minor groove of DNA. This property of polyamines suggests that they might be involved in DNA stability and conformation, and may be related to gene expression. It should be noted that these are only hypotheses at present. This property may be responible for the capacity of polyamines to enhance the action of many drugs used in cancer chemotherapy. In plants polyamines have been shown to affect cell membranes, such that the membranes are stabilized by their addition. Polyamines may be useful in the culture of plant protoplasts, plant cells that have had their cell walls enzymatically removed, due to this fact. Their involvement in a plant's response to osmotic and pH stress is also probably related to effects on cell membranes.

On a physiological level, polyamines may act as second messengers of plant growth regulators. The action of auxins, cytokinins, gibberelins, ethylene as well as phytochrome all appear to involve elevations or reductions (ethylene) of intracellular polyamine levels.

One of the most interesting aspects of polyamine metabolism is their role in embryonic animal development and differentiation of tissues. When administered to pregnant mice in their drinking water, inhibitors of polyamine biosynthesis were found to not only reduce polyamine levels, but to block embryo development as well. The contragestational effects of polyamine inhibitors were also confirmed in two other mammals, rats and rabbits. The findings that polyamines are important in embryo development have been extended to other groups of animals, as the experimental reduction of cellular polyamines was later found to block embryo development in birds (chickens) and amphibians.

Plant growth and development are also greatly influenced by intracellular levels of polyamines. Elevated levels of polyamines have been correlated to periods of growth in a number of whole plant and tissue culture systems. Polyamine levels increase 20% in cultured *Helianthus* tissue when growth is induced, and a similar trend has been noted in suspension cultured tobacco tissue. Other studies have shown that polyamine synthesis increases during a specific phase of the cell cycle and it has been suggested that polyamines stimulate progression through the cell cycle.

As has been demonstrated in animals, polyamines also play important roles in the organized development of plant tissues. A number of plant tissues are ideally suited for the study of developmental processes, including actively growing meristems found at shoot or root tips. As dormant buds on potato tubers begin to grow and elongate, polyamines and the enzymes leading to their biosynthesis rise dramatically. A gradient of polyamine concentration and enzyme activity is established, with higher levels being found in the shoot tip (meristem). Levels decline as the distance from the meristem increases. Temporal changes in the levels of polyamines and related enzymes occur in a number of plant developmental systems. Elevations are associated with embryonic development of tobacco, soybean and rice, with highest levels of polyamines being found primarily in the ovule or developing embryos. In developing rice seeds, for example, polyamines and ADC are significantly elevated 12-16 days after fertilization of the flower, coinciding with stages of embryo development. Similar trends are observed in developing tobacco and soybean seeds.

As was the case in animal studies, the most convincing evidence to support the hypothesis that polyamines play a central role in plant development, and are not merely associated with or are the unrelated effects of organized growth, involves the use of the polyamine biosynthesis inhibitors. Intracellular levels of the polyamines can be experimentally manipulated with these compounds. In developing tomato ovaries, for example, dramatic elevations of polyamines and the enzyme ODC are associated with fruit and seed development. ODC levels rise over 4-fold in these tissues after pollination. Inhibition of ODC in those tissues blocked fruit and seed development. The same results have been found using other widely divergent groups of plants. Fluctuations in levels of polyamines correlate to embryo formation in the forest trees aspen and red pine, and inhibitors of polyamine synthesis again reduced seed/embryo formation in those species.

Tissue cultured plant cells can very easily be studied and manipulated, and have proven valuable in determining the role of polyamines in plant growth and development. Tissues from nearly every group of plants can be grown in culture, either on agar or in liquid suspensions, and differentiation of these cells occurs in a large number of species. Cells in carrot suspension cultures, for example, can be induced to form embryos by simply changing the plant growth regulators in the culture medium. The advantages of using tissue cultures include the facts that large numbers of embryos are formed nearly synchronously, enviornmental conditions are easily controlled, tissue is available on a year round basis, and the effects of experimental compounds on cell growth and development can easily be studied. In tobacco cultures, elevations of polyamines were observed when shoots or roots were formed, adding more evidence to the hypothesis that polyamines are associated with plant development. More conclusive results were obtained by the use of the polyamine inhibitors in tissue cultures, however. Levels of both polyamines and enzymes responsible for their synthesis rise when embryo development is induced in carrot cultures. DFMA, an inhibitor of the enzyme ADC, was found to block this rise in cellular polyamines, and more importantly embryogenesis was dramatically reduced in these cultures. When polyamines were added to the culture medium along with the DFMA, embryogenesis proceeded as in the untreated control cultures. It was possible, evidently, to fulfill the polyamine requirement by adding polyamines to the medium. These results strongly suggest that polyamines are not merely associated with, but may be essential to embryonic development in carrot. Similar results have been obtained with aspen, in which the inhibition of polyamine synthesis led to reduced shoot formation in the cultures.

From the evidence currently at hand it appears that polyamines strongly influence or control plant growth and development. The physiological, biochemical and molecular mechanisms by which the polyamines exert their effects are not clear, but are being studied in a number of laboratories around the world. The fact that polyamines modulate growth and development in both plants and animals suggests that they may affect a fundamental or basic aspect of development. It is exciting that at least certain aspects of growth and development are common in plants and animals. This may provide valuable insights into our understanding of development in both of these groups of organisms.

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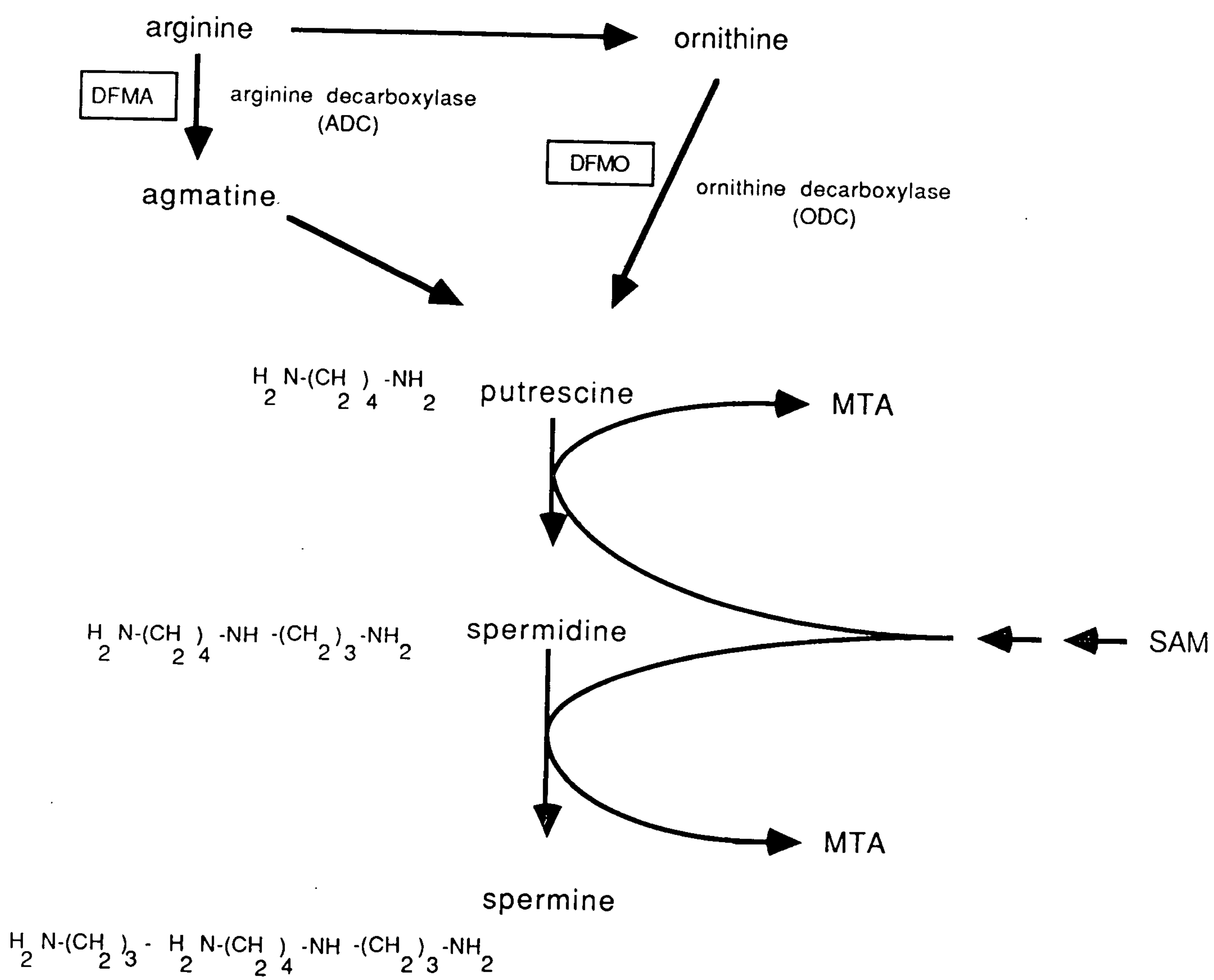


Figure 1. Summary of polyamine biosynthesis. Enzyme inhibitors indicated by boxes.